ACTA BIOLOGICA SZEGED. 24 (1–4), pp. 39–51 (1978)

RAPID DETERMINATION OF DROUGHT-RESISTANCE OF NEW RYE, MAIZE AND LUPINE VARIETIES WITH THE LIVE-WILTING PROLINE TEST

G. PÁLFI, J. NÉMETH, L. PINTÉR, KATALIN KÁDÁR and W. BÖLKE

Department of Plant Physiology, Attila József University Szeged;
Research Institute for Cereal Production, Szeged;
VEG (Z) Saatzucht Bornhof, Saatzuchtstiation Bocksee, DDR.

(Received May 1, 1977)

Abstract

In six varieties of proline-type rye, in two of non-proline-type maize, and in five of lupine, apart from measurement of the proline content of the water-deficient, isolated shoots, the concentrations of the free total amino-acid and of the soluble total protein were also determined. The proline concentration of the water-deficient isolated shoots turned out to be a positive correlation with the amount of the total amino-acid and the soluble total protein, and with the degree of drought-resistance. The new complex method, after some further experiments, may therefore be suitable for evaluating the drought-resistance of new varieties of plants.

It also turned out that, as a result of a water-deficiency of the same degree in the isolated shoots, no qualitative difference could be demonstrated in the free amino-acid compositions of plants belonging to the same species. However, whereas the amount of proline predominates among the free amino-acids of the proline-accumulating species (*e.g.* rye), among the non-proline-type species (*e.g.* maize and lupine) the asparagine concentration may achieve the highest level.

Introduction

It was established in our previous work that, as a result of a strong water-deficiency, the essential amino-acids, asparagine and particularly proline accumulate very considerably in the green shoots. At the same time, the total amount of the free protein-producing amino-acids also increases to two to three times as much as in the (non-water-deficient) control (PÁLFI, 1968a b). In the course of investigations into capsicum, tobbaco, wheat, barley, and rye plants, it turned out that the drought-resistance of the individual varieties is proportional to the amount of free proline accumulated in the leaves (PÁLFI, 1969; PÁLFI and JUHÁSZ, 1971; KUDREV, 1970; LEWITT, 1972; SINGH and ASPINALL, 1972). On the basis of quantitative measurements of the proline content in the leaves of plants suffering from a water-deficiency, a simple procedure was elaborated in order to decide the drought-resistance of the individual varieties the new method being named the "proline test" (PÁLFI, 1969; PÁLFI and JUHÁSZ, 1971; PÁLFI et al., 1973).

It was also established that, as a result of a water-deficiency and live-wilting conditions of the same degree, quite different proline amounts accumulate in the different plant species.

Starting from all these data, the cultivated soft-stalk plants were divided into two groups: 1 = proline-type species; 2 = non-proline-type species (PÁLFI et. al.,

1974a b; 1975a b). A species is named of proline-type if it is live-wilted isolatedly, *i. e.* the free proline content of the shoots or leaves suffering from a strong water-deficiency reaches or exceeds 10 mg in 1 g dry matter (1.0 per cent of the dry matter).

Below this quantity the species is no longer of proline-type. From among fifty cultivated soft-stalk plants only seventeen nonproline-type species were found. The majority of the cultivated plant species are therefore of proline type (PÁLFI et al., 1974a b).

In the present experiment we have striven to clarify how, apart from proline, the total amount of the free protein-forming amino-acids and the concentration of the soluble total protein develop in the course of live-wilting of the isolated shoots and leaves, inducing a strong water-deficiency.

Can this qualitative difference be demonstrated as a result of the strong water-deficiency in the free amino-acid composition of the isolated shoots of varieties belonging to the same species?

To study the problem, we investigated altogether twelve varieties of two plant species of non-proline-accumulating type (lupine and maize) and of one species of proline-type (rye).

In the course of the experiments, two varieties of hybrid maize were applied as guides; the drought-resistances of these are well-known, because the results of culture experiments during several years have shown a considerable, one-direction deviation in this respect (PINTÉR et al., 1976).

Materials and Methods

The new rye (*Secale cereale L.*) and Yellow lupine (*Lupinus luteus L.*) varieties were improved by M. BRUMMUND and W. BÖLKE in a loose sandy soil, in "VEG Saatzucht Bornhof, Saatzuchtstation Bocksee" (Kreis Waren, DDR). The production and preservation of the hybrid maize varieties (*Zea mays L.*) was performed by JÁNOS NÉMETH in Szeged, in an adobe field soil, in the establishment of the Cereal Research Institute.

The names (symbols) and stages of development of the varieties investigated are as follows:

Rye: From three sorts of rye, shoot samples were taken immediately before flowering. The variety symbols are: R_1, R_{33}, R_{81}.

The shoots of the other three rye varieties were collected in the phase of flowering. The symbols are: R_{131}, R_{132}, R_{139}.

Lupine (Lupinus): On budding, two varieties of lupine shoot samples were taken, their names being: 1 = Bornhof; 2 = Refusanova. The names of the other three varieties of lupine taken on flowering are: 1 = Afus; 2 = Borluta; 3 = Pilaf = (Bocksee).

Maize: Two varieties were investigated, but in three phases of development. The two hybrid maize varieties are:

1. BcSK-5/a; its symbol: W64A×Oh43. In Jugoslavia it is a variety recognized by the State and in Hungary it is allowed for raising. It demands water and is only slightly drought-resistant.

2. KSC.360; its symbol: A90×153R. It is a Hungarian-improved variety, recognized by the State. It needs only little water. According to the experimental results of PINTÉR et al., (1976), it is much more drought-resistant than the previously-mentioned Jugoslav variety. For the two maize varieties full shoots were isolated twice for live-wilting (at the ages of seven and eleven leaves), while on the third occassion only leaves were detached and processed (from among the 14-leaf shoots the eleventh leaves counted from below).

The average result was the live-wilting of 20 isolated shoots each of the rye and lupine varieties and 10 of maize. The shoot groups were put on trays according to varieties and the live-wilting was performed at a temperature between 20 and 28 °C. The relative air humidity was 60 to 70 per cent, and the constant illumination 2000 lux. The isolation of rye and lupine shoots lasted for two days and that of maize for four days. (The live-wilting of isolation

leaves was carried out only for three days). During this time, the water content of the shoots had to be reduced by 60 to 70 per cent.

The analyses were performed from the pulverized matter of the plants fixed (and dried) immediately on being detached, and after being live-wilted at 70 °C.

For analysis of amino-acids, 200 mg dry matter was homogenized with 1 g quartz sand, in a mortar, with 20 ml 30 per cent ethanol, and leached twice out of the mortars dishes into centrifuge tubes from the residue of the 20 ml. The homogenizate was purified by being centrifuged. Finally, as a result of evaporation and wetting losses, we obtained 18 ml extract. This was the starting basic solution quantity for all analysis and concentration calculations.

The method of measuring the free total amino acid with a universal standard was published earlier (PÁLFI et al., 1972).

In proline determinations a phenol-water (4:1) mixture was used as solvent. After isatin development (Figs. 3 and 4) and the eluation of spots, the extinctions were measured with a spectrophotometer.

The soluble total protein originating from the leaves of the live-wilted shoots was measured by nephelometry, according to COLOWICK and KAPLAN (1957); with some modifications. From 0,2—0,5 g living material, the proteins of the extracts (0,5 or 1,0 ml) made with 20 ml Tris buffer of pH 7,5 were reacted with 4 ml portions of three kinds of precipitating solutions:

1) 5% trichloracetic acid; *2)* 2,5% sulphosalicilic acid; *3)* 0,7% $K_4Fe(CN)_6$ + 0,3 ml concentrated acetic acid.

By variation of the quantity of the starting protein-extracts, the turbidity is adjusted so that the 4 ml reagent should induce only the mild, homogeneous opalescence of the solution. Agglutination, precipitation and fast deposition may not occur. The extract amount tested with trichloracetic acid is generally optimum for the other two protein precipitants, as well.

For the calibration curve, a standard series of "Bovine blood serum albumin" (5, 10, 15, 20 and 30 mg) was measured out and dissolved in 20 ml Tris, and the extinctions of the solutions were measured.

The results of three repetitions were averaged per reagent, and the final result was obtained after the partial results achieved in this way were averaged again.

If the mean error in the average results for repetitions of identical samples was larger than ±5 per cent, then the whole analysis was repeated.

Results and discussion

First we wanted to clarify whether there is any qualitative difference in the composition of free amino-acids as a result of the strong water-deficiency provoked by live-wilting the isolated shoots of the improved varieties belonging to the same species. Of the prepared chromatograms, an 18-strip paper developed with ninhydrin is demonstrated, involving the extracts of three varieties of lupine, three of rye, and two of maize (Fig. 1). In the evaluation of the chromatogram, it is also to be taken into consideration that only the growth and live-wilting of the varieties belonging to the same species took place at the same time, together and under identical conditions (a real result is therefore only obtained in the case of comparing the varieties belonging to the same species).

It is to be seen in Fig. 1 that no qualitative difference was induced in the composition of the free amino-acids of the varieties belonging to the same species, as a result of a water-deficiency of the same degree. An essential quantitative difference was found only in the proline and asparagine concentrations of the individual species. Most proline is contained in the rye varieties (a species of proline type), and asparagine is mostly accumulated in the lupine and maize varieties. According to CREACH et al., (1974) the large amount of asparagine in the maize plant acts as an internal carbon dioxide reserve, although maize is overwhelmingly "malat-forming". In respect of the chromatogram in Fig. 1 is to be noted that if the extent and colour

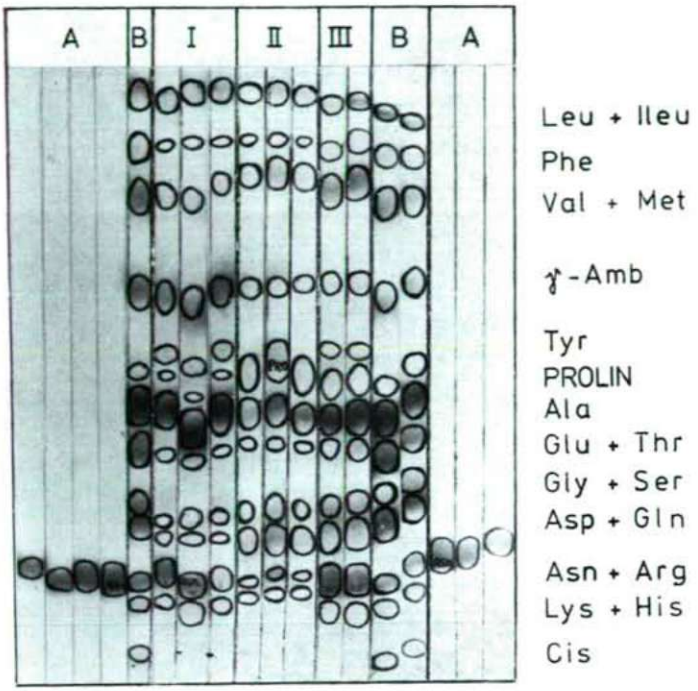

Fig. 1. Qualitative composition of free amino acids of the Yellow lupine, rye, and maize varieties as a result of live-wilting connected with a strong water-loss. The live-wilting of the lupine and rye varieties took place with isolated shoots, but with isolated leaves for the maize varieties. Ascending chromatogram. Solvent: butanol-acetic acid-water (3:1:1). Ninhydrin development, fixing with copper salt.
A = Asn standards: 5, 10, 15, 20, 30, 20, and 10 µg;
B = Universal standards with 30, 45 and 30 µg total amino acid content;
I = Yellow lupine varieties: Afus, Borluta, and Bocksee;
II = Rye varieties: R_1, R_{33}, and R_{st};
III = Hybrid maize: W64A × Oh43 (foreign) = BcSK 5/a;
A90 × 153R (Hungarian variety) = KSC 360.

intensity of the asparagine spots of the extracts are compared with those of the standards, then an approximative evaluation is possible concerning the absolute quantity.

The question may arise, however, as to whether the varieties run on a single strip can give evaluative results. As regards this question, it must be taken into consideration that the varieties of all species were run on a separate chromatogram, too, on 3 to 4 adjacent strips, *i.e.* in 3 to 4 repetitions. In Fig. 2 a chromatogram is demonstrated in which the extracts two varieties of Yellow lupine were developed in a uniform quantity, in four adjacent repetitions. At the edges of the paper, the universal standard appears in three different quantities.

It turns out from Fig. 2 that, if identical quantities of an extract made from the same variety are run in four repetitions, we may obtain uniform separations and spot sizes. The method is therefore reliable. The further important conclusion at

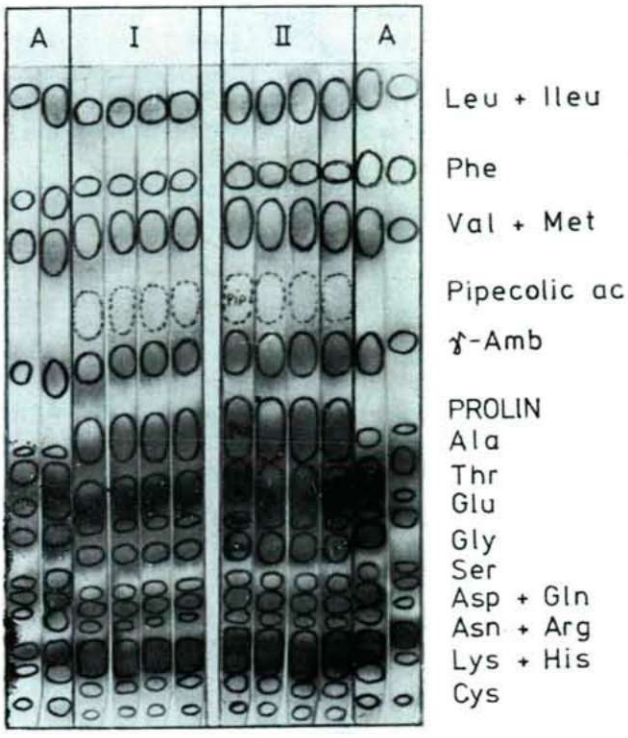

Fig. 2. Qualitative composition of free amino acids of the two lupine varieties, as a result of the live-wilting of the isolated shoots, connected with a strong water-loss. The chromatogram was made with the same butanol solvent and ninhydrin development as recorded in the previous Figure. The extracts of both varieties were run on four adjacent strips, *i. e.* in four repetitions.
A = Universal standards, in sequence, from the left: 15, 30, 45 and 30 µg total amino-acid content;
I = Bornhof variety (4 repetitions);
II = Refusanova variety (4 repetitions).

present is that no qualitative difference is to be observed between the amino-acid compositions of the two varieties.

It is also to be seen in the chromatogram that the spots of the "Refusanova" variety (the four repetitions on the right) are generally somewhat larger than those of the "Bornhof" variety, on the left. The total amino-acid concentration of the "Refusanova" variety is therefore larger. In Fig. 2, a cyclic amino-acid is also present: pipecolic acid, which is not a protein-former, but is characteristic of legumes.

As regards Fig. 1 and 2, it may be stated that, if the proline is developed with ninhydrin, they only give spots of pale (light yellow) colour. Further, this spot cannot be eluted, either to be measured colorimetrically. In the quantitative measurement of proline, we have therefore applied the highly sensitive isatin, which forms an intense, dark-blue spot with proline. In Fig. 3 a chromatogram is visible which was developed with isatin for measurement of proline quantities. It illustrates the extracts

of three varieties of rye, in three repetitions each. In addition, proline standards as well were run on the same paper.

In Fig. 3 it is discernible that, from among the free amino-acids of rye varieties, proline gives dark (blue) spots of very considerable size (rye being a species of proline-accumulating type) and, if a phenolic solvent is applied, it is fully separated from the other amino-acids. From the chromatogram it is visible even to the naked eye that the proline and total amino-acid concentrations of the rye variety run in the middle (R_{33}) are higher than those of the other two varieties. It is also seen from the chromatogram that — apart from proline — the other amino-acids form only pale spots of very little extent, as they respond poorly to the isatin developer.

In the following, we shall examine what spot intensity is given by proline, and what is the quantity and relation of the other amino-acids, as compared with proline, in the case of the phenolic running on paper of the extracts of lupine varieties belonging to the non-proline type, and of their isatinic development (Fig. 4). It must be mentioned in advance, however, that in the case of varieties belonging to the non-proline type — *e.g.* lupine — it is necessary to apply (3 to 4 times as much extract as from the varieties of proline type) for the proline to appear in a measurable quantity.

It turns out from Fig. 4 that in the case of the extracts of the isolatedly live-wilted, that is to say strongly water-deficient shoots of lupine-varieties, the largest

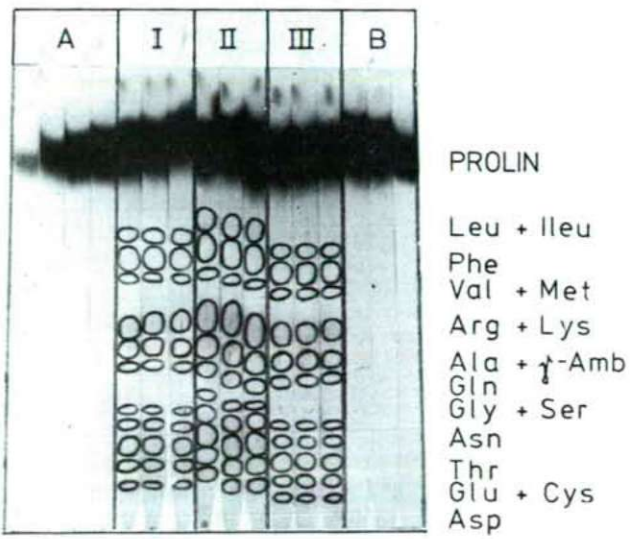

Fig. 3. Separation of the free amino-acids of the rye varieties belonging to the proline-accumulating type, and the quantitative determination of proline in the case of applying a phenol-water (4:1) solvent. Developer: isatin solution, which reacts strongly with proline. Identical quantities of all varieties were run on three adjacent strips, i. e. in three repetitions. The strong water-deficiency of the varieties was induced by live-wilting the isolated shoots for two days.
A and B = proline standards, starting in sequence from the left: 5, 10, 15, 20; and 40, 30, 20 μg;
I = rye variety R_1; II = rye variety R_{33}; III = rye variety R_{st}.

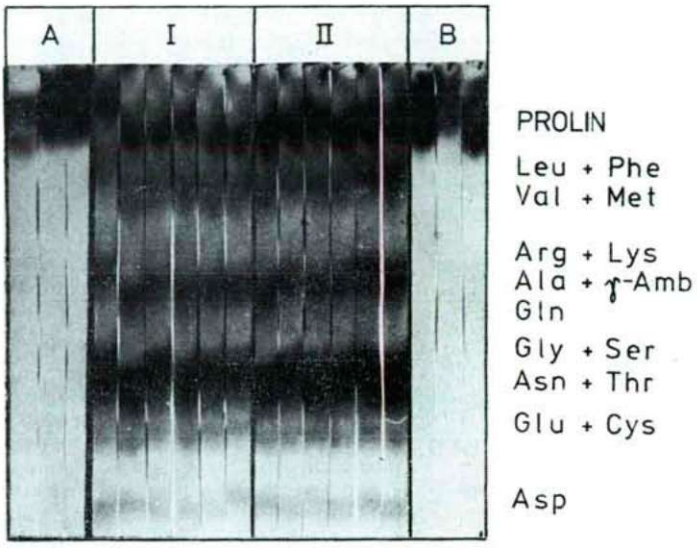

Fig. 4. Determination of the proline in the two varieties of Yellow lupine of non-proline-type, with a paper-chromatographic, eluation method. Live-wilting took place with isolated shoots. Identical quantities of the extracts of the lupine varieties were run on six adjacent strips each, i.e. repeated six times. The solvent was phenol-water, and the developer was isatin, as in the previous Figure. On both sides of the chromatogram, on the three extreme strips, proline standards were run.
A and B = proline standards in sequence from the left: 5, 10, 15; and 20, 5, 10 μg;
I = Bornhof variety; II = Refusanova variety.

and most intensive spot is not given by proline; it is a common spot of asparagine + + threonine. It was established by ATKINS et al., (1975) that in the case of lupine and most legumes the most N-containing important product of nitrogen-fixation and nitrate reduction is asparagine. In addition, asparagine similarly acts as a primary N-source of protein synthesis. Proline does not always predominate, therefore, among the free amino-acids of the plant species not belonging to the proline type. At any rate, in this method, if the optimum quantity of extract was applied, the amino-acids are still reasonably separeted and proline is present in a quantity that can be eluted well for colorimetric measurement.

It is also to be seen in Fig. 4 that proline is contained in a major amount by the extract of the "Refusanova" variety (strip 6, on the right on the paper).

Before reporting the results of our quantitative measurements, it is to be mentioned that for any variety of every species, on isolating a group of shoots (control), we immediately measured the fresh matter. After being fixed, this was then dried and, after live-wilting for two, three or four days, the wilting weights were measured too. It was established from these weights whether live-wilting could be finished, as the wather-deficiency had already achieved 65 to 70 per cent compared with the fresh weights. After starting from an identical weight of fresh matter, as well as of live-wilted matters of the same water-loss, the dry-matter contents were also measured for all varieties. It was established that, as regards the dry-matter contents measured in this way, we obtained no characteristic difference

according to variety, whether we had started from fresh or from live-wilted matter. The free amino-acid contents related to 1 g dry matter give, therefore, a correct basis for comparison. Taking into consideration that samples were taken from the two hybrid maize varieties three times, primarily the data of these are reported here (Table 1).

The provocation of the water-loss of the isolated full shoots was carried out in the case of maize, with live-wilting from the third till the sixth day. However, even during the six days, the reduction of the water-content in the isolated shoots did not reach 65 to 70 per cent, but amounted only to 55 per cent. At the same time, the largest free amino-acid and total-protein contents were achieved in the shoots live-wilted for four days. These data are therefore given in Table 1.

Table 1. The free proline, total amino acid and soluble total protein contents of the isolated shoots and leaves of two hybrid maize varieties, as a result of a strong water-loss (live-wilting). At the 7- and 11-leaf ages, the full shoots were isolated; their live-wilting, inducing a strong water-deficiency, lasted with constant illumination for four days. At the 14-leaf age, the eleventh leaves were isolated, counted from below, and their live-wilting lasted for three days. A real basis for comparison is only given by the results of the varieties equally developed, raised under identical conditions, and live-wilted together, (varieties separated from each other by horizontal lines in the Table)

Hybrid maize	Development of shoots at the time of isolation	Proline content from the live-wilted sample; mg/g dry mater	Total amino-acid content, mg/g dry matter		Soluble total protein; live-wilted matter mg/g
			From shoots		
			fixed on detaching	live-wilted	
Hungarian hybrid KSC 360 A90×153R	7-leaf shoots	1,1	9,9	60,3	39,6
Foreign hybrid BcSK 5/a W64A×Oh43	7-leaf shoots	1,2	9,0	52,7	33,1
Hungarian hybrid KSC 360 A90×153R	11-leaf shoots	1,2	20,4	71,1	45,8
Foreign hybrid BcSk 5/a W64A×Oh43	11-leaf shoots	1,1	20,4	60,6	37,5
Hungarian hybrid KSC 360 A90×153R	isolated leaves	3,9	16,8	49,8	42,9
Foreign hybrid BcSK 5/a W64A×Oh43	isolated leaves	3,2	14,4	41,0	35,2

It is clear from Table 1 that, in the course of the live-wilted water-loss of the isolated full shoots, in the case of maize, at the times of both the 7- and 11-leaf developments only a very small proline amount was accumulated. In addition, there was no characteristic difference between the proline contents of the two varieties investigated, either. Therefore, in the course of sampling 3, we have no longer isolated full shoots, but only leaves (counting leaf 11 from below in the shoots with 14 leaves). In the course of live-wilting the isolated leaves, we have already achieved a 70 per cent water-loss in three days, as compared with the water-content of the freshly-isolated leaves.

It can be established from Table 1 that, as a result of this strong water-loss of the isolated leaves, the proline concentration became three times higher as compared with the isolated shoots (1.2 : 3.9). At the same time, there is a considerable difference between the proline contents of the varieties as well. The Hungarian improved variety, *i.e.* "KSC 360" accumulated 21.8 per cent more proline than the variety of foreign origin, "BcSK 5/a".

This result at all events supports our previous finding (PÁLFI et al.,1973, 1974a, b) that maize is not a proline-accumulating type because, even as a result of a strong water-deficiency the proline concentration of the leaves does not amount, to 10 mg/g dry matter.

It is to be seen from Table 1 that in the free total amino-acid contents of shoot- and leaf-samples fixed and dried immediately after being detached, there is hardly any characteristic difference between the two varieties. However the total amino-acid amounts of the samples taken at the times of different developments differ from each other uniformly and strongly in both varieties. The total amino-acid content of the first isolated 7-leaf shoot sample for instance, is more than two times lower than that of the shoot sample taken on the second occasion. Further, as compared to this, the total amino-acid content of the isolated leaves is even smaller (by 25 to 30 per cent). However, we are mainly interested in the total amino-acid content, formed primarily as a result of the live-wilting water-loss of the isolated shoots.

Table 1 shows that the amount of the free total amino-acids accumulated as a result of the strong water-deficiency, at all three developmental and sampling-stages was larger for the Hungarian variety "KSC 360" than for the foreing variety "BcSK 5/a" (the difference falls between 14.4 and 21.4 per cent).

In Table 1, it is demonstrated that in the course of analysing the live-wilted matter of a strong water-loss, in respect of the soluble total protein, too, for all three samplings the larger amounts were given by the Hungarian improved variety as compared with the foreign one (the difference falls between 19.6 and 22.1).

It can therefore be established from the data of Table 1 that the free total amino-acid and soluble total-protein contents of the variety showing a stronger proline concentration as a result of the strong water-deficiency, i.e. the Hungarian "KSC360", also achieved considerably higher levels than those of the foreign variety, "BcSK 5/a". As the drought-resistances of both maize varieties investigated are already known from the results of raising experiments (PINTÉR et al., 1976), it can be stated that the amounts of the free total proline, total amino-acid, and soluble total protein at higher level, accumulated as a result of the strong water-loss, are connected with the higher degree of drought-resistance.

In estimating the drought-resistances of maize varieties, we can also consider

that, in the course of isolating, live-wilting, and analysing the young but already fully-developed leaves, taken from the upper one-third of shoots in the state of development before tasselling, a major proline content can be achieved and that the difference between the varieties may be more considerable.

It was established by JOVANOVIC (1975) that the crop of hybrid maize was most influenced by the amount of precipitation in the period lasting from the 9-leaf stage till tasseling. This result supports our suggestion that, in order to determine drought-resistance, the sample taken in the phase before the development of tasselling is most suitable because the maize plant is most sensitive to a water-deficiency at this time.

GUPTA and KOVÁCS (1974) put in an air-conditioning plant for four days maize plants raised under glass in a culture-vessel until their 5-leaf stage and induced in these a strong water-deficiency. They then set out the seedlings in the field and, during the raising of the plants, determined their drought-resistance. The results agree with our finding that the considerable differences during the determination are given by the maize plants exposed to a strong water-deficiency for four days.

In the following, we pass to an analysis of the rye and lupine varieties. In an evaluation of the results of Table 2, however, it is to be taken into consideration that real data for comparison are given only by the analysed data of the live-wilted shoots isolated at the same time from among the shoots raised under identical cultural conditions, belonging to the same species and showing the same development. The data corresponding to these conditions are separated from one another by horizontal lines drawn between species and varieties. Correspondingly, the rye and lupine varieties at the two stages of development form separate groups in respect of evaluation (Table 2).

In Table 2, the first three, *i. e.* the free proline, total amino-acid and soluble total protein contents of rye variety R_{33}, for the isolated shoots, live-wilted for earing, resulted in much higher values than the other two, varieties, R_1 and R_{st} (R_{st} = standard variety). At the same time, the higher-level proline and total amino-acid contents of variety R_{33} are also reflected by the chromatogram of Fig. 3. Similarly, one of the three rye varieties, R_{132}, showed a considerably higher level, in respect of both proline and total amino-acid and soluble total protein. The other two rye varieties R_{131} and R_{139}, afforded fairly uniform concentrations as compared with each other concerning the materials investigated.

Similarly to the analyses of maize varieties, therefore, it can also be stated for the rye samples that the same variety gave characteristically higher values in regard to the total amino-acid and the soluble total protein, as well, in which the proline concentration of the isolated shoots rose to a higher level during the strong water-loss. It can be established from the proline amounts of the rye varieties that the rye species belongs to the proline-accumulating type: as a result of a strong water-deficiency, for all the six varieties investigated, the proline concentration of their isolated shoots exceeded the critical 10 mg/g level.

SINGH and ASPINALL (1972) investigated the drought-resistances of ten barley varieties, belonging similarly to the proline type. The rapid formation of a strong water-deficiency was induced by wetting for three days the root medium of the plants raised in a culture-vessel for a period of three weeks with a hypertonic solution of polyethylene glycol (-20 bar). SINGH and ASPINALL demonstrated, as a result of the strong water-deficiency of barley at the same level, very different proline concentrations in the leaves (from 9.0 up to 24.1 mg/g dry matter) according to varieties.

Table 2. The free proline, total amino-acid and soluble total protein contents of the isolated shoots of the rye and Yellow lupine varieties, as a result of a strong water-loss (live-wilting). Live-wilting lasted for two days with constant illumination. (Only the developments of the species and varieties separated from each other by horizontal lines are identical.)

Species and varieties	Development of shoots at the time of isolation	Proline content from the live-wilted sample; mg/g dry matter	Total amino-acid content, mg/g dry matter		Soluble total protein; from live-wilted matter mg/g
			From shoots		
			Fixed on detaching	live-wilted	
Rye: R_1	on earing	15,2	11,2	23,6	27,6
Rye: R_{33}	on earing	22,8	13,5	32,5	33,5
Rye: R_{81}	on earing	12,5	11,6	24,3	27,9
Rye: R_{131}	on flowering	12,7	12,7	24,6	25,7
Rye: R_{132}	on flowering	20,3	15,0	30,9	31,4
Rye: R_{139}	on flowering	11,5	12,8	23,8	25,8
Lupine Bornhof	on budding	3,4	16,3	47,2	36,7
Lupine Refusanova	on budding	4,8	16,8	52,8	44,2
Lupine Afus	on flowering	3,5	15,4	39,7	35,5
Lupine Borluta	on flowering	4,2	15,9	45,6	41,8
Lupine Bocksee	on flowering	3,0	15,1	34,2	32,1

The authors proved with their field-growing experiments with the same ten barley varieties that drought-resistance of the varieties increases in direct proportion to the rise in the proline concentration. This fact has already been ascertained by us in our former investigations and by other authors, as well (PÁLFI, 1969; PÁLFI and JUHÁSZ, 1971; PÁLFI etal., 1973; ASPINALL et al., 1973; WALDREN and TEARE, 1974).

Returning to the data of Table 2, it is clear that from among the two kinds of lupine samples taken on gemmation, the proline, total amino-acid, and soluble total protein concentrations of the Refusanova variety show higher values than those of the Bornhof variety. These results can also be concluded from the spot sizes of the chromatograms in Fig. 4.

In Table 2., the results of the three kinds of lupine varieties taken on flowering show a gradual differenciation: the proline, total amino-acid and soluble total protein concentrations of the Bocksee variety are the smallest. Those of the Afus variety are already higher, but those of the Borluta variety achieved the highest level. The results on isolated lupine shoots, taken on budding and flowering and exposed

to a strong water-deficiency, similarly to the data obtained for the rye varieties, indicate that if the proline concentration of a variety is higher then the total amino-acid and soluble total protein amounts of the same variety rise to a higher level, as well.

ASPINALL et al., (1973), BATES et al., (1973), LEWITT (1972), SINGH and ASPINALL (1972) and WALDREN and TEARE (1974) determined the degree of drought-resistances of plants on the basis of the concentration of accumulated free proline. They did not take into consideration whether the plants investigated were of proline-accumulating type or not. This new classification has not been applied by other authors apart from ourselves as yet.

It is to be seen from the results (Tables 1 and 2) that the isolated shoots of the species classified into the non-proline type accumulated only a relatively small quantity of proline as a result of a strong water-deficiency. In cases like this, the possibility of errors in analyses increases. It seemed to be advisable to determine, not only the proline, but also the free total amino-acid and soluble total protein contents which amounted, as a result of the water-deficiency, to 200 to 300 per cent of the control well supplied with water. According to our results, not only the amount of free proline accumulated owing to the water-deficiency is in correlation with the drought-resistance of the variety investigated, but also the free total amino-acid and soluble total protein concentrations if live-wilting was carried out for the required time and under the optimum conditions. Therefore, after being further investigated, this complex method seems to be suitable for the rapid evaluation of the droght-resistances of the new plant varieties created by the improvers.

References

ASPINALL, D., SINGH, T. N., PALEG, L. G. (1973): Stress metabolism. V. Abscisic acid and nitrogen metabolism in barley and *Lolium temulentum* L. — Austral. J. Biol. Sci. *26*, 319—327.

ATKINS, C. A., PATE, J. S., SHARKEY, P. J. (1975): Asparagine metabolism — key to the nitrogen nutrition of developing legume seeds. — Plant Physiol. *56*, 807—812.

BATES, L. S., WALDREN, L. P., TEARE, I. D. (1973): Rapid determination of free proline for water stress studies. — Plant and Soil. *39*, 205—207.

COLOWICK, P. S., KAPLAN, N. O., (1957): Methods in enzymology. Vol. III. Acad. Press Inc. Publishers, New York.

CREACH, E., MICHEL, J. P., THIBAULT, P. (1974): Aspartic acid as an internal CO_2 reservois in *Zea mays*. — Planta (Berl.) *118*, 91—100.

GUPTA, D., KOVÁCS, I., (1974): A method of measuring drought resistance in maize and drought resistance of parents, single, 3-way and double crosses of Opaque-2 maize inbreds and their normal analogues. — Z. Pflzücht. Berlin—Hamburg *73*, 125—130.

JOVANOVIČ, R., (1975): Proucavanje uticaja meteoroloskih uslova, dubine obrade, kolicine NPK hraniva i velicine hranivog prostora na prinos kukuruza i neke fizicke osobine zemlista. — Arh. Poljoprivr. Nauke. Beograd. *28*, 123—134.

KUDREV, T. G. (1970): On the problem of the formation of proline under the effect of drought. — Dokl. Acad. Selhoz. Nauk v Bulg. *3*, 261—264.

LEWITT, J. (1972): Responses of plants to environmental stresses. New York. Acad. Press.

PÁLFI, G. (1968a): Die Wirkung von Kinetin, 2,4-DNP und Antimetaboliten auf die Veränderungen im Aminosäurengehalt welkender Pflanzenblättern. — Planta (Berl.) *78*, 196—199.

PÁLFI, G. (1968b): Changes in the amino acid content of detached wilting leaves of *Solanum laciniatum* AIT. in the light and in the dark. — Acta Agron. Acad. Sci. Hung. *17*, 381—388.

PÁLFI, G. (1969): Az öntözés szükségességének a megállapítása és a szárazságtűrő növényfajták kiválogatása új, gyors módszerrel (Establishment of the necessity of irrigation and selection of drought-resistant plant varieties with a new, rapid method). — Növénytermelés (Budapest) *18*, 25—34.

PÁLFI, G., BITÓ, M., SEBESTYÉN, R. (1972): Néhány levélzöldség és takarmánynövény szabad aminosav tartalmának fokozása élve-hervasztással. (Increasing the free amino acid content of some herbage leaves and fodder-plants with live-wilting). — Növénytermelés (Budapest) *21*, 203—214.

PÁLFI, G., BITÓ, M., ZSÓFIA PÁLFI. (1973): Svobodniy prolin i vodniy deficit rastitelnikh tkaney. — Fiziol. Rasteniy, Moskva. *20*, 233—238.

PÁLFI, G., JUHÁSZ, J. (1971): The theoretical basis and practical application of a new method of selection for determining water deficiency in plants. — Plant and Soil. *34*, 503—507.

PÁLFI, G., ERZSÉBET KÖVES, BITÓ, M., SEBESTYÉN, R. (1974a): The role of amino acids during water-stress in species accumulating proline. — FITON (Argentina) *32*, 121—127.

PÁLFI, G., ERZSÉBET KÖVES, NEHÉZ, R. (1974b): A vízhiányban szenvedő kultúrnövények aminosav szabályozásának két fő típusa és a gyakorlati mezőgazdaságban való alkalmazása. — Növénytermelés (Budapest) *23*, 219—228. (Two main types of amino acid regulation of water-deficient cultivated plants and their application in practical agriculture).

PÁLFI, G., ERZSÉBET KÖVES, NEHÉZ, R. (1975a): Effect of growth-substances on the free amino-acid content of lentil shoots in case of water deficiency. — Acta Biol. Szeged. *21*, 69—82.

PÁLFI, G., KUDREV, T. G., NEHÉZ, R. (1975b): Az absciszinsav és az indolecetsav hatása a lucernahajtások aminosav anyagcseréjére vízdeficit esetén. — Növénytermelés (Budapest) *24*, 339—349. (Effect of abscissic acid and indole-acetic acid on the amino acid metabolism of lucerna shoots in the case of water deficiency).

PINTÉR, L., KÁLMÁN, L., NÉMETH, J., PÁLFI, G., (1977): Két eltérő vízigényű kukoricahibrid izolált hajtásaiban a szabad prolin-, az össz-aminosav- és oldható össz-fehérje tartalom vizsgálata. — Növénytermelés (Budapest) *26*, 253—263 (Investigation into the free proline, the total amino-acid and soluble total protein contents in isolated shoots of two maize hybrids of different water needs).

SINGH, T. N., ASPINALL, D. (1972): Proline accumulation and varietal adaptability to drought in, barley: a potential metabolic measure of drought resistance. — Nature New Biology *236*, 188—189.

WALDREN, R. P., TEARE, I. D. (1974): Free proline accumulation in drought stressed plants under laboratory conditions. — Plant and Soil *40*, 689—692.

Address of the authors:

Dr. G. PÁLFI
Department of Plant Physiology,
A. J. University
H-6701 Szeged, P. O. Box 428,
Dr. J. NÉMETH, Dr. L. PINTÉR
and KATALIN KÁDÁR
Research Institute
for Cereal Production
H-6701 Szeged, P. O. Box 391,
Dr. W. BÖLKE
VEG (Z) Saatzucht Bornhof,
2061 Bocksee, Post Ankershagen
(Kr. Waren), DDR.